

Applicant : David White et al.
Serial No. : 09/804,357
Filed : March 12, 2001
Page : 6

Attorney Docket No.: 07334-109002

REMARKS

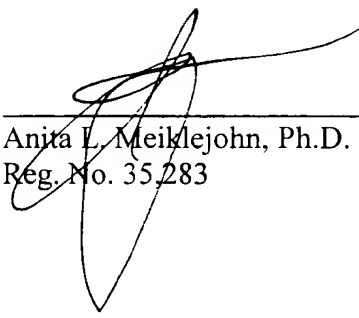
This amendment is made solely to insert the enclosed corrected Sequence Listing into the specification and to correct sequence identifiers in the specification. No new matter is introduced.

Attached is a marked-up version of the changes being made by the current amendment.

Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 15 OCT 2001



Anita L. Meiklejohn, Ph.D.
Reg. No. 35,283

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

Version with markings to show changes made

In the specification:

Paragraph beginning at page 17, line 16, has been amended as follows:

Figure 2 depicts a series of alignments of the amino acid sequence of LIG46 (SEQ ID NO:2) with portions of a number of galactosyltransferases, including (from top to bottom): *Mus musculus* UDP-Gal: betaGlcNAc beta 1,3-galactosyltransferase-I (Accession Number AF029790; SEQ ID NO:[] 8); *Mus musculus* IPP-Gal: betaGlcNAc beta 1,3-galactosyltransferase-III (Accession Number AF029792; SEQ ID NO:9); *Drosophila melanogaster* neurogenic secreted signalling protein ("Brainiac"; Accession Number U41449; SEQ ID NO:[] 10); and *Homo sapiens* UDP-galactose: 2-acetamido-2-deoxy-D-glucose3beta-galactosyltransferase (Accession Number Y15014; SEQ ID NO:[] 11). The amino acid sequence above the solid line is a majority sequence (SEQ ID NO:12).

Paragraph beginning at page 18, line 12, have been amended as follows:

Figure 7 depicts the cDNA sequence of human LIG46 (SEQ ID NO:13).

Paragraph beginning at page 18, line 13, has been amended as follows.

Figure 8 depicts the predicted amino acid sequence of human LIG46 (SEQ ID NO:14).

Paragraph beginning at page 18, line 15, has been amended as follows.

Figure 9 depicts an alignment of the cDNA sequences of human LIG46 (upper sequence; SEQ ID NO:13) and murine LIG46 (lower sequence; SEQ ID NO:1).

Paragraph beginning at page 18, line 18, has been amended as follows.

Figure 10 depicts an alignment of the predicted amino acid sequences of human LIG46 (upper sequence; SEQ ID NO:14) and murine LIG46 (lower sequence; SEQ ID NO:2).

Paragraph beginning at page 19, line 20, has been amended as follows:

A nucleotide sequence encoding murine LIG56 protein is shown in Figure 4 (SEQ ID NO:[5]4; SEQ ID NO:[7]6 includes the open reading frame only). A predicted amino acid sequence of LIG46 protein is also shown in Figure 4 (SEQ ID NO:[6]5).

Paragraph beginning at page 19, line 24, has been amended as follows.

The murine LIG56 cDNA of Figure 4 (SEQ ID NO:[5]6) encodes a 400 amino acid protein.

Paragraph beginning at page 93, line 15, has been amended as follows:

The LIG46 cDNA isolated as described above (SEQ ID NO:1) has a 1191 nucleotide open reading frame ([nucleotides __ - __ of SEQ ID NO:1;] SEQ ID NO:3) which encodes a 397 amino acid protein (SEQ ID NO:2). This protein includes a predicted signal sequence of about 32 amino acids (from amino acid 1 to about amino acid 32 of SEQ ID NO:2) and a predicted mature protein of about 365 amino acids (from about amino acid 33 to amino acid 397 of SEQ ID NO:2; SEQ ID NO:4). The extracellular domain of LIG46 extends from about amino acid 33 to about amino acid 302. LIG46 protein possesses one predicted transmembrane domain which extends from about amino acid 303 (extracellular end) to about 320 (intracellular end) of SEQ ID NO:2. The cytoplasmic domain of LIG46 extends from about amino acid 321 to about amino acid 397.

Paragraph beginning at page 94, line 18, has been amended as follows:

Portions of LIG46 are similar to certain galactosyltransferases. Figure 2 depicts a series of alignments of portions of the amino acid sequence of LIG46 (SEQ ID NO:2) with portions of a number of galactosyltransferases, including: *Mus musculus* UDP-Gal: betaGlcNAc beta 1,3-galactosyltransferase-I (Accession Number AF029790; SEQ ID NO:[]8); *Mus musculus* IPP-Gal: betaGlcNAc beta 1,3-galactosyltransferase-III (Accession Number AF029792; SEQ ID NO:9); *Drosophila melanogaster* neurogenic secreted signalling protein (Accession Number U41449; SEQ ID NO:[]10); and *Homo sapiens* UDP-galactose: 2-acetamido-2-deoxy-D-glucose3beta-galactosyltransferase (Accession Number Y15014; SEQ ID NO:[]11). A

majority sequence is depicted above the solid line (SEQ ID NO:12). Conserved residues are shaded. These residues are more likely conserved in functional variants of LIG46.

Paragraph beginning at page 95, line 5, has been amended as follows:

Figure 7 depicts the cDNA sequence of a full-length human LIG46 clone. Figure 8 depicts the predicted amino acid sequence of human LIG46. The human LIG46 cDNA depicted in Figure 7 (SEQ ID NO:[]13) has a 1191 nucleotide open reading frame which encodes a 397 amino acid protein (SEQ ID NO:[]14). This protein includes a predicted signal sequence of about 32 amino acids (from amino acid 1 to about amino acid 32 of SEQ ID NO:[]14) and a predicted mature protein of about 365 amino acids (from about amino acid 33 to amino acid 397 of SEQ ID NO:[]14; SEQ ID NO:[]15). Figure 9 depicts an alignment of the cDNA sequences of human LIG46 (upper sequence) and murine LIG46 (lower sequence). Figure 10 depicts an alignment of the predicted amino acid sequences of human LIG46 (upper sequence) and murine LIG46 (lower sequence).

Paragraph beginning at page 96, line 6, has been amended as follows:

Briefly, the LIG46 gene was mapped using the Genebridge 4 Radiation Hybrid panel. A pair of primers within the 3' untranslated region of LIG46 (forward-CCATGTTGGGGTCTCACATTAGAG, SEQ ID NO:[]18; and reverse-GGTAAGTCAGACCAATATCCTGCC, SEQ ID NO:[]19) were used to amplify DNA from the Genebridge 4 panel. The PCR products were run on a 2% agarose gel, stained with SYBR Gold and scanned. Linkage analysis was performed using the Map Manager QT623 software package.

Paragraph beginning at page 98, line 12, has been amended as follows:

For this study, a phosphothioate-protected antisense oligodeoxynucleotide and its respective control sequence (sense) were synthesized. The antisense oligodeoxynucleotide targets the LIG46 start codon mRNA at position 39.

Antisense: 5' CTT CGA CGC CCC ACA CTC AT 3' (SEQ ID NO:[] 16)

Applicant : David White et al.
Serial No. : 09/804,357
Filed : March 12, 2001
Page : 10

Attorney, Docket No.: 07334-109002

Sense: 5' ATG AGT GTG GGG CGT CGA AG 3' (SEQ ID NO:[] 17)

Male obese *ob/ob* C57BL/6J (45 g) mice were individually housed in macrolon cages ($22 \pm 2^\circ \text{C}$; 12:12 h light/dark cycle with lights off at 6 pm). Tap water and mouse chow diet were given *ad libitum*. Mice were stereotactically implanted with a chronic guide cannula aimed to the third ventricle (intracerebroventricular) one week prior to this experiment.